The genus *Rubus* in South Africa. III. The occurrence of apomixis and sexuality

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The genus *Rubus* L. contains two ovules per ovary, one above the other. The embryo sac developing in the upper ovule degenerates. The lower ovule contains either aposporic or sexual embryo sacs. In some species degeneration of sexual embryo sacs was observed. With the exception of *R. apetalus* Poir., apospory is absent in the subgenus *Idaeobatus* Focke. Since all South African *Rubus* species have sexual embryo sacs, with the exception of *R. flagellaris* Willd. where all sexual embryo sacs eventually degenerate, they have the potential to act as pollen receptors during hybridization. The morphological differences between the embryo sacs of different species are not sufficient to distinguish between species.

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Die genus *Rubus* L. bevat twee saadknoppe per vrughok. Die twee saadknoppe is bo-op mekaar geleë. Die kiemsak in die boonste saadknop degenereer altyd. Die onderste saadknop kan geslagtelike of aposporiese kiemsakke bevat. In sommige spesies is degenerasie van geslagtelike kiemsakke waargeneem. Met uitsondering van *R. apetalus* Poir., is aposporie onbekend in die subgenus *Idaeobatus* Focke. Aangesien alle Suid-Afrikaanse spesies van die genus *Rubus* geslagtelike kiemsakke bevat, met uitsondering van *R. flagellaris* Willd. waar sodanige kiemsakke mettertyd degenereer, besit hulle die potensiaal om as moederplante in kruisings op te tree. Die morfologiese verskille tussen die kiemsakke van verskillende spesies is te min om tussen die spesies te onderskei.

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Introduction

The occurrence of polyploidy and apomixis in the genus *Rubus* L. has resulted in the formation and recognition of many microspecies (Beijerinck 1953). By 1913 approximately 3350 different taxa had been described in the genus (Gustafsson 1943). These taxa include localized varieties and primary hybrids (Gustafsson 1943).

It is known that various reproductive systems occur in the genus *Rubus* (Dowrick 1966). Cytological evidence of sexual reproduction, as well as obligate and facultative apomixis, has been presented (Christen 1950; Dowrick 1961). In addition to reproduction by means of seed, vegetative reproduction occurs frequently (Heslop-Harrison 1959; Kirby 1980). The complexity of the reproductive system in the genus is further increased by the occasional occurrence of diplosporic and aposporic embryo sacs within the same individual and even in the same ovary (Christen 1950; Czapik 1981a, 1981b). It is generally assumed that the subgenus *Idaeobatus* Focke contains only sexually reproducing representatives (Heslop-Harrison 1953).

For taxonomical purposes it is essential to know whether the *Rubus* species occurring in South Africa represent true species or hybrids. In order to obtain this information, the hybridization potential of each taxon must be determined. The aim of this study was, therefore, to determine whether sexual or apomictic reproduction occurs in the South African *Rubus* populations, and to determine whether apomixis, if present, occurs as apospory, diplospory or both.

Materials and Methods

The voucher material used in this study has previously been listed (Spies & Du Plessis 1985). Young inflorescences were fixed and stored in Navashin's fixative (Stockholm modification) (Maheshwari 1939) at 4°C. The flowers were dissected in 30% ethanol in order to separate the different ovaries. Ethanol and tertiary butanol were used for dehydration and the ovaries were embedded in a synthetic wax (Tissue Prep T565). Sections (5 µm) were prepared and mounted by using the method described by Jensen (1962).

Embryo sacs of *Rubus* tend to overstain with most published methods. The safranin and fast green double staining technique of Johansen (1940) and Sass (1951) was modified and used for this study in the following way: the wax was removed in xylene (2 changes — 10 min each). Subsequently preparations were taken through xylene/ethanol (50:50), absolute ethanol and 70% ethanol for 5 min each and stained overnight in safranin (100 ml ethanol, 100 ml water,

4 g sodium acetate and 8 ml 40% formalin added to 4 g safranin dissolved in 200 ml methyl cellosolve). The slides were rinsed in running water until all excess safranin was removed. Slides were destained in picro – ethanol (0,5 g picric-acid in 100 ml ethanol) for 2 min. Preparations were thereafter passed through ammonia – alcohol (3 – 4 drops ammonium hydroxide in 100 ml ethanol) and absolute ethanol (1 min in each). Counterstaining was done with fast green (0,33 g fast green in 100 ml ethanol) for 15 sec and subsequently destained in absolute ethanol; two changes — the first for 15 sec and the second until the preparation was sufficiently destained when observed with a microscope. The preparations were mounted in Eukitt after the slides had been rinsed in an ethanol/xylene mixture (50:50) and in two changes of xylene (5 min each). Between 14 and 40 embryo sacs per plant were studied.

Results

The *Rubus* carpel encloses two ovules, one located above the other (Figures 1a & 2a). Both ovules are anatropous, unitegmentic and crassinucellate. The difference in ontogenetic development between the upper and lower ovules varies between different species. Usually the embryo sac in the upper ovule develops slower than the lower one and eventually aborts (Figures 2b – d). Contrary to the usual situation in the Angiosperms, the young *Rubus* ovules contain a central group of elongated archesporia (Figure 2e). However, these cells are not clearly defined and they gradually merge into the surrounding nucellular cells. One of these archesporial cells divides meiotically and forms four megaspores, one of which develops into an eight-nucleate *Polygonum*-type embryo sac (Figures 1b – f). Deviations from this general pattern will be discussed separately for each species.

Aposporic embryo sac development implies the development of at least one diploid somatic nucellus cell into an embryo sac instead of a haploid sporogenic cell. Although the mature aposporic embryo sac morphologically resembles the *Polygonum*-type embryo sac in the genus *Rubus*, it is relatively easy to distinguish between them. The only degenerating tissue during sexual embryo sac development is the degenerating three non-functional megaspores at the micropylar pole of the developing embryo sac. During aposporic development the aposporic embryo sac crushes the tetrad megaspores or the developing embryo sac. This degenerating tissue can be observed at both poles and on the side of the developing embryo sac and is observed by its darkly stained appearance with no distinguishable cellular contents

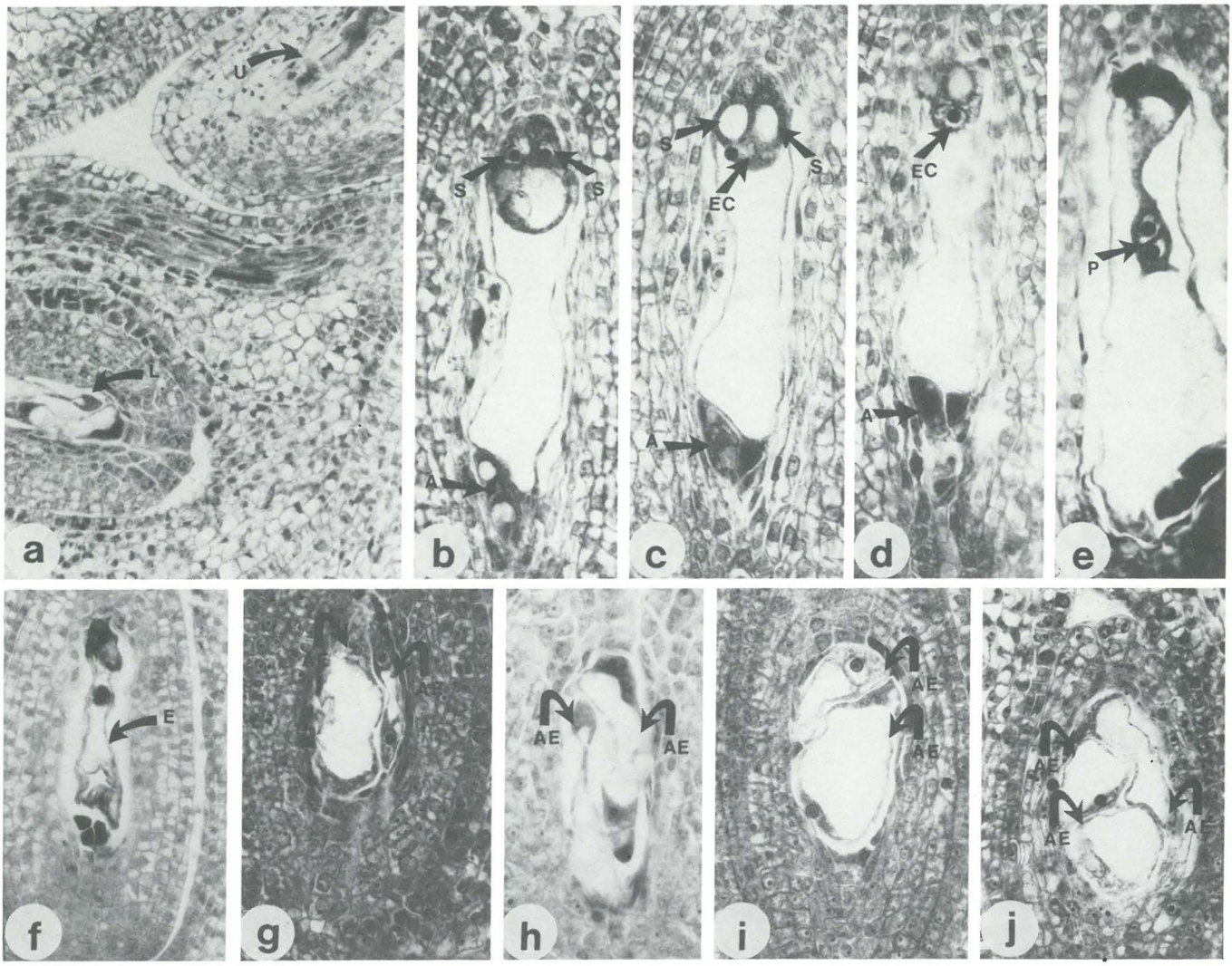

Figure 1 Macrosporogenesis in the genus *Rubus*. **(a)** Orientation of the upper (U) and lower (L) ovules in a longitudinal section — sexual upper (U) and aposporic lower embryo sacs (L) (*R. flagellaris — Henderson & Gaum 2*); **(b – f)** mature sexual embryo sacs [(b – d) *R. longepedicellatus* (*Henderson & Gaum 36*); (e) *R. apetalus* (*Henderson & Gaum 1*); (f) *R.* × *proteus* (*Henderson & Gaum 28*)]; **(g – j)** aposporic embryo sacs [(g & i) *R.* × *proteus* (*Stirton 9866*); (h) *R. flagellaris* (*Henderson & Gaum 2*); (j) *R. pascuus* (*Stirton 9868*)].
A, antipodal cells; AE, aposporic embryo sac; E, sexual embryo sac; EC, egg cell; L, lower ovule; P, polar nuclei; S, synergid; U, upper ovule. (×1150).

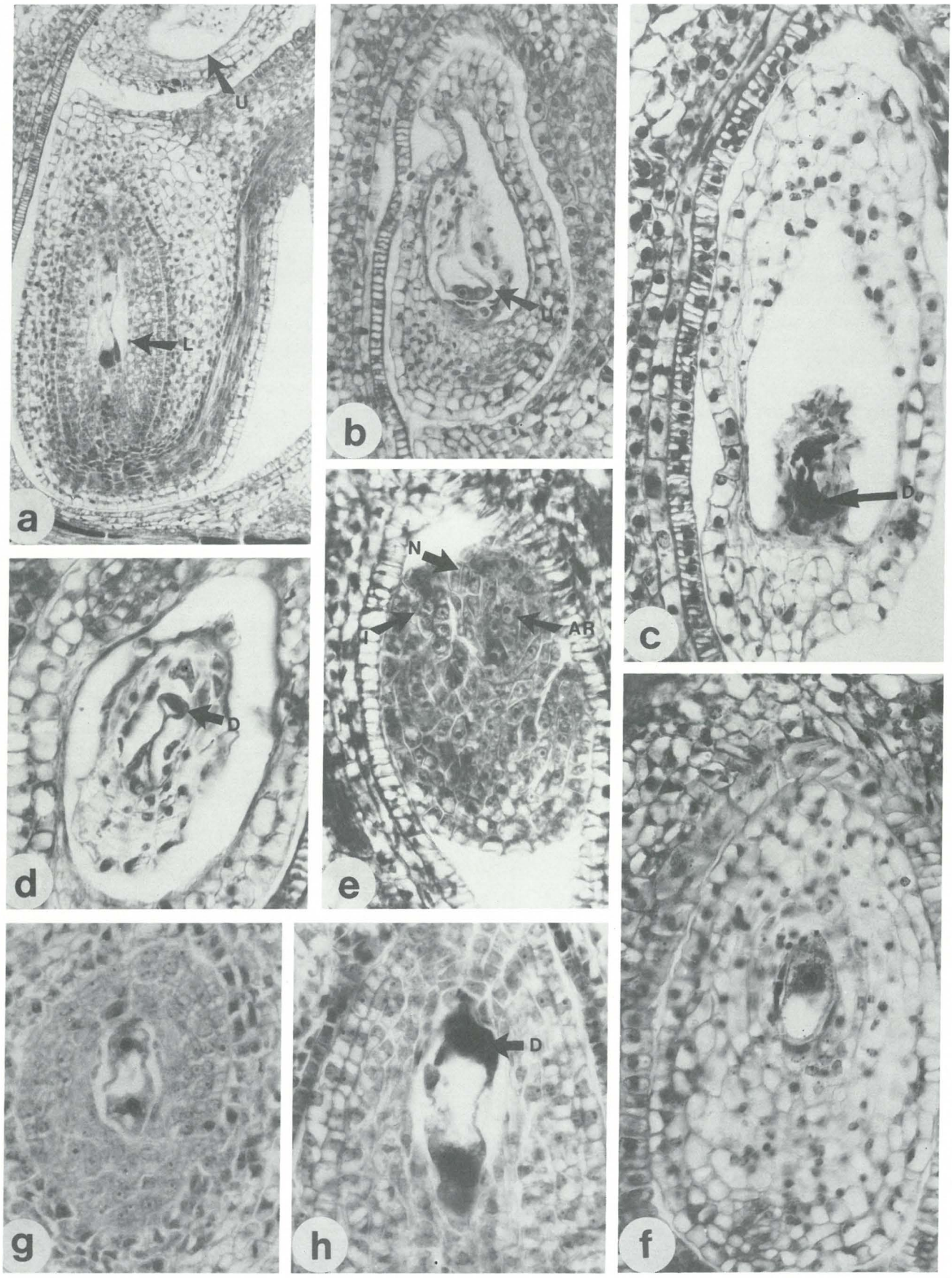

Figure 2 Macrosporogenesis in the genus *Rubus*. **(a)** Orientation of upper and lower ovules in a longitudinal section (*R.* × *proteus* — *Henderson & Gaum 28*); **(b – d)** degeneration of embryo sacs in the upper ovules [(b) *R.* × *proteus* (*Henderson & Gaum 28*); (c) *R.* × *proteus* (*Stirton 9798*); (d) *R. longepedicellatus* (*Henderson & Gaum 36*)]; **(e)** archesporium (*R. pascuus* — *Stirton 9868*); **(f)** uni-nucleate embryo sac (*R. pascuus* — *Stirton 9800*); **(g)** bi-nucleate embryo sac (*R. pascuus* — *Henderson & Gaum 18*); **(h)** degenerating tetra-nucleate embryo sac (*R. cuneifolius* — *Stirton 8102*).
AR, archesporium; D, degenerating tissue; I, integument; L, lower ovule; N, nucellus; U, upper ovule. (a, ×800, b – h, ×1400).

(Figure 2c & h). In addition to this difference between aposporic and sexual embryo sacs, the number of embryo sacs per ovule can also indicate which type of embryo sac is present. In contrast to the single sexual embryo sac observed in sexually reproducing plants, more than one embryo sac usually occurs in aposporic plants (Figure 1g – j).

Subgenus *Eubatus* Focke

This subgenus includes four exotic species in South Africa (Spies & Du Plessis, 1985) of which the following three were included in this study:

R. cuneifolius Pursh.

Initiation of embryo sac development in the upper ovule is later than in the lower one. The chalazal megaspore or the second megaspore from the chalazal side further develops into mature *Polygonum*-type embryo sacs. The majority of embryo sacs degenerate after the four-nucleate stage (Figure 2h). This degeneration occurs in both the upper and lower embryo sacs. The developing aposporic embryo sac crushes the tetrad. Apospory was observed in only one specimen, *Henderson & Gaum 93*, where it occurs in 55% of the cells studied (Table 1).

R. pascuus L.H. Bailey

The upper embryo sac develops much later than the lower one. The lower one usually reaches maturity, while the upper one is still in the tetrad stage. Except for the differences in developmental stage between the upper and lower embryo sacs, no other differences have been observed. Both upper and lower ovules contain either sexual or

Table 1 Percentage of sexual, aposporic and degenerating sexual embryo sacs in various *Rubus* species

Voucher No.	2n	Sexual	Aposporic	Degenerating	Total number of cells studied
Rubus cuneifolius					
Stirton 8102	14	53,13	–	46,87	32
Liengme s.n.	21	75,0	–	25,0	24
Henderson & Gaum 93	28	45,0	55,0	–	20
R. pascuus					
Henderson & Gaum 18	21	46,15	46,15	7,69	13
Stirton 9800	21	35,0	20,0	45,0	20
Stirton 9861	28	100,0	–	–	20
Stirton 9868	28	35,0	65,0	–	20
R. flagellaris					
Henderson & Gaum 2	28	62,5	34,38	3,13	32
R. apetalus					
G. Hemm s.n. a	14	100,0	–	–	20
G. Hemm s.n. b	28	88,89	11,11	–	18
Henderson & Gaum 6	28	75,0	12,5	12,5	24
Wells 5000	28	100,0	–	–	20
Henderson & Gaum 1	–	100,0	–	–	20
R. longepedicellatus					
Henderson & Gaum 22	14	54,17	–	45,83	24
Henderson & Gaum 14	28	50,0	–	50,0	28
Stirton 9862	28	50,0	–	50,0	40
Henderson & Gaum 36	35	65,0	–	35,0	20
Henderson & Gaum 30	–	85,0	–	–	20
Wells 5001	–	90,0	–	10,0	20
R. pinnatus					
Henderson & Gaum 15	14	50,0	–	50,0	34
R. ludwigii					
Henderson & Gaum 41	14	56,52	–	43,48	23
R. × proteus					
Henderson & Gaum 28	14	68,18	–	31,82	22
Stirton 9866	21	4,76	95,24	–	21
Stirton 9798	28	–	100,0	–	20
Henderson & Gaum 27	28	100,0	–	–	25
Stirton 9865	35	6,67	–	93,33	15
Henderson & Gaum 20	35	68,18	–	31,82	22
Henderson & Gaum 31	42	50,0	–	50,0	26
Stirton 8135	49	–	–	100,0	20
Henderson & Gaum 50	56	67,86	28,57	3,57	28
Stirton 9869	–	35,71	35,71	28,57	14
R. transvaliensis × R. longepedicellatus					
Henderson & Gaum 10	28	100,0	–	–	18

aposporic embryo sacs.

Sexual and aposporic embryo sacs are present in the triploid plants (Figures 1j & 2e – g) (Table 1). However, the sexual embryo sacs degenerate either at maturity or earlier. Among the tetraploid plants, *Stirton 9861* is 100% sexual, whereas only 35% of the embryo sacs of *Stirton 9868* are sexual (Table 1).

R. flagellaris Willd.

The only specimen studied, *Henderson & Gaum 2*, contains both sexual and apomictic embryo sacs (Table 1). The lower and upper embryo sacs develop almost simultaneously (Figure 1a). The upper embryo sac is usually short and round, whereas the lower one is much longer. The upper ovule usually contains a sexual embryo sac, whereas the lower one contains an aposporic embryo sac (Figures 1a & h). This is the only specimen in which sexual and aposporic embryo sacs have been observed within a single ovary. Both embryo sacs in the upper and lower ovules reach maturity, whereafter the upper sexual embryo sac degenerates.

Subgenus *Idaeobatus* Focke

In South Africa this subgenus is represented by nine indigenous and two exotic species (Spies & Du Plessis, 1985). Only four indigenous species and a hybrid swarm formed by hybridization of *R. pascuus* (subgenus *Eubatus*) and *R. longepedicellatus* (subgenus *Idaeobatus*) were examined in this study.

R. apetalus Poir.

The lower embryo sac develops before the upper one. Apospory occurs in two of the specimens studied, i.e. *G. Hemm s.n. b* and *Henderson & Gaum 6*, (Table 1). However, the percentage of aposporic embryo sacs is very low (11,1% and 12,5% respectively). All mature sexual embryo sacs show signs of degeneration (Figure 1e). The nucellus usually degenerates during the early stages of embryo sac development in *G. Hemm s.n. b* and *Henderson & Gaum 6*. This results in the formation of an embryo sac surrounded by integumental cells.

R. longepedicellatus (C.E. Gust.) C.H. Stirton

In all specimens studied the upper embryo sacs degenerate before the two-nucleate stage (Figure 2d). Apospory does not occur at any ploidy level (Table 1). Only 16,7% of the sexual embryo sacs in the lower ovule of the diploid specimen, *Henderson & Gaum 22*, reach maturity. The rest degenerate after the four-nucleate stage. No degeneration of the lower embryo sacs has been observed in the tetraploid specimens, *Henderson & Gaum 14* and *Stirton 9862* (Figures 1b – d).

Degeneration of the nucellus usually occurs in *Henderson & Gaum 14* (the nucellus degenerates in 85,7% of the ovules). The morphology of these embryo sacs corresponds with that of *R. apetalus*. Although the pentaploid specimen, *Henderson & Gaum 36*, apparently forms only sexual embryo sacs, they all degenerate at maturity.

R. pinnatus Willd.

Only sexual embryo sacs are formed in the specimen studied (Table 1). All the upper embryo sacs degenerate. The nucellus usually degenerates at an early stage. In cases where normal sexual development occurred, the embryo sacs are very elongated. Although a large number of embryo sacs in the lower ovules degenerate, developing embryos are sometimes present, thus indicating that not all embryo sacs degenerate.

R. ludwigii Eckl. et Zeyh.

Only one specimen, *Henderson & Gaum 41*, was studied (Table 1). In all the ovaries the embryo sacs in the lower ovules develop into sexual embryo sacs, whereas the upper ones degenerate. The development of the upper embryo sacs is initiated much later than in the lower ovules.

R. × proteus

This taxon represents a hybrid swarm between *R. pascuus* and *R. longepedicellatus*. The diploid specimen, *Henderson & Gaum 28*, contains sexual embryo sacs. However, all the upper, and the majority of the lower embryo sacs degenerate before maturity (Figures 2b & c). *Stirton 9866*, the triploid specimen, contains 4,8% sexual embryo sacs which degenerate (Table 1). Aposporic embryo sacs occur in both the lower and the upper ovules (Figures 1g & i). The tetraploid specimen, *Henderson & Gaum 27*, reproduces sexually, whereas only aposporic embryo sacs occur in *Stirton 9798* (Table 1). Although both pentaploid specimens *(Stirton 9865* and *Henderson & Gaum 20)* produce sexual embryo sacs, they all degenerate. In the hexaploid specimen, *Henderson & Gaum 31*, the upper and all but two of the lower sexual embryo sacs degenerate. All embryo sacs of the heptaploid specimen, *Stirton 8135*, degenerate. The octoploid specimen, *Henderson & Gaum 50*, contains both sexual and aposporic embryo sacs (Table 1). Although the majority of embryo sacs are sexual (71,4%), they all degenerate and, therefore, this specimen reproduces apomictically.

The sexual embryo sacs in the upper ovules degenerate before the two-nucleate stage. The mature embryo sacs vary from short (Figure 1f) to very elongated.

R. transvaliensis × R. longepedicellatus

Although the specimen studied, *Henderson & Gaum 10*, is an interspecific hybrid, embryo sac development reveals the normal development of sexual *Polygonum*-type embryo sacs. Initiation of development of the lower embryo sac precedes that of the upper one. The upper embryo sac usually degenerates.

Discussion

Apomixis is the process where sexual reproduction is replaced by various types of asexual reproduction. This process may either involve vegetative reproduction (Heslop-Harrison 1959; Kirby 1980) or agamospermy (Thomas 1940; Einset 1951; Pratt & Einset 1955; Dowrick 1961, 1966; Czapik 1981a, 1981b). This involves the asexual reproduction of embryos and seeds. Although both agamospermy and vegetative reproduction are present in the genus *Rubus*, this study concentrated on agamospermy.

The results of this study indicate that agamospermy, in the form of apospory, is present in the three species of the subgenus *Eubatus* studied. However, differences occur within the species. The two tetraploid specimens of *R. pascuus* vary from 0% apospory (*Stirton 9861*) to 65% apospory (*Stirton 9868*). The results show that the subgenus *Eubatus* has 75,1% sexual and 24,9% aposporic embryo sacs. Although these figures seem to indicate the prevalence of sexuality, sexual reproduction is much less prevalent. The sexual embryo sacs,

formed in the triploid specimens of *R. cuneifolius* and *R. pascuus*, degenerated, as did all the embryo sacs in the upper ovules of the diploid specimens. All mature sexual embryo sacs in *R. flagellaris* indicated degeneration. Therefore, this species reproduces only apomictically. When these facts are taken into consideration, a maximum of 24,3% sexual embryo sacs in the subgenus *Eubatus* will reach maturity and will be fertilized. Therefore, there is only a small difference in the number of sexual embryo sacs that will reach fertilization (24,3%) and aposporic embryo sacs formed (24,9%). Both *R. cuneifolius* and *R. pascuus* can be considered as facultative apomicts, whereas *R. flagellaris* may be considered as an obligate apomict because all the sexual embryo sacs degenerated.

Apospory was present in only two specimens of the subgenus *Idaeobatus*. Both specimens, *G. Hemm s.n. b* and *Henderson & Gaum 6*, belonged to *R. apetalus*. Since it is generally assumed that the subgenus *Idaeobatus* is only composed of sexually reproducing representatives (Heslop-Harrison 1953), this example is the only known deviation. Degeneration of sexual embryo sacs occurred in all the uneven polyploid levels. These plants are, therefore, entirely dependent on vegetative reproduction. The degeneration of sexual embryo sacs in *R. apetalus* suggests an abnormality, possibly interspecific hybridization. However, meiotic analysis of anther squashes indicate that both *G. Hemm s.n. b* and *Henderson & Gaum 6* are segmental alloploids (Spies *et al.* 1985) with normal meiosis. It is, therefore, not clear why this abnormality occurs during megasporogenesis.

The diploid *R. longepedicellatus* specimen, *Henderson & Gaum 22*, exhibits embryo sac degeneration in 16,7% of the ovules studied. This phenomenon may be attributed to the fact that a number of univalents were observed in the anthers during meiosis (Spies *et al.* 1985) and that a similar phenomenon might occur during megasporogenesis. The tetraploid specimens behaved as normal alloploids, therefore the formation of the univalents may be due to either hybridization or haploidization. The degeneration of embryo sacs in the pentaploid specimen indicates that an abnormal segregation of chromosomes during meiosis may be responsible for nuclear death and consequently for embryo sac degeneration.

Markarian & Olmo (1959) suggested that the degeneration of the upper ovule typifies the genus *Rubus*. The results of this study support their suggestion. Although the degeneration or abortion of the upper ovule may occur at different developmental stages, all upper ovules eventually abort.

No cytogenetical evidence for the degeneration of the majority of embryo sacs in the lower ovules of the diploid *R. pinnatus* specimen could be obtained from a meiotic analysis of microsporogenesis (Spies *et al.* 1985).

Another interesting feature observed during this study is the degeneration of the nucellus. This phenomenon was observed in *R. apetalus, R. longepedicellatus* and *R. pinnatus*. The degeneration of the nucellus leads to the degeneration of the embryo sac (Pratt *et al.* 1958).

The results of this study support those of Pratt *et al.* (1958) who reported both sexual and apomictic embryo sacs in triploid *Rubus* plants. Their results indicated 25,6% apospory with a resultant seedset of ± 30%, whereas this study indicates that 38,5% of the embryo sacs formed by triploids are aposporic. The low seedset may be attributed to the seemingly sexual embryo sacs that degenerate at a later stage. Therefore, a large number of ovules do not contain any functional embryo sacs.

Since sexual embryo sacs are formed in almost all the species studied, they all have the potential to hybridize. The only exception is *R. flagellaris*, where all sexual embryo sacs degenerated and in which only apospory occurs. The higher frequency of sexual embryo sacs observed in the subgenus *Idaeobatus*, indicates that this subgenus has a higher potential to participate as female parent in hybridization than species belonging to the subgenus *Eubatus*.

Conclusions

The results of this study indicate that both sexual and apomictic reproduction is present in the *Rubus* species occurring in South Africa. Apospory is the only form of apomictic reproduction observed in the genus. Apospory is excluded from the majority of *Idaeobatus* species (with the exception of *R. apetalus*). However, the differences in the frequencies of sexual and aposporic embryo sacs vary within a species.

The occurrence of sexual embryo sacs in all the species, with the exception of *R. flagellaris* where all sexual embryo sacs degenerate before maturity, indicate that all species have the potential to act as pollen receptors during hybridization. This information regarding the hybridization potential of the different *Rubus* species will provide a useful tool in the specific delimitation of the genus *Rubus*. However, the morphological features of the embryo sacs of different species differ slightly and these differences are not sufficient to distinguish between species. It is, therefore, concluded that hybridization is theoretically possible in this genus but no information regarding the delimitation of species could be obtained. However, the combination of these results and other cytogenetic and morphological studies will be sufficient to distinguish between true species and hybrids.

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